The effects of temperature and senescence on the accumulation of reducing sugars during storage of potato (*Solanum tuberosum* L.) tubers: A mathematical model

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Abstract

A dynamic mathematical model based on underlying physiological processes is developed to describe, analyze and predict the storage behaviour of potato (*Solanum tuberosum* L.) tubers in terms of accumulation of reducing sugars. The data necessary for calibration and validation of the model were gathered during long term storage experiments over a wide range of storage temperatures for several seasons and cultivars. Although the model is based on a considerable simplification of the occurring physiological processes, it is capable of accounting for about 95% of the storage behaviour, including both cold-induced and senescent sweetening. The concept is postulated that the state of maturity at time of harvest determines storage behaviour through the initial amount of enzyme (or enzyme system) responsible for cold-induced sweetening.

1. Introduction

In potatoes, reducing sugars are involved in the non-enzymatic browning reaction, known as the Maillard reaction (Ashoor and Zent, 1984; Ellis, 1959; O'Brien and Morrissey, 1989), and thus the amount of reducing sugars (glucose and fructose) determines the processing potential of potatoes in terms of frying colour (Brown et al., 1990; Burton, 1989; Fuller and Hughes, 1984; Marquez and Añon, 1986; Pritchard and Adam, 1994; Yada et al., 1985). The amount of reducing sugars in potatoes at the

time of processing depends on the conditions during the preceding storage period (Burton, 1989).

In storage, a tuber is not a static entity, and during storage a constant flow of carbohydrates is released from starch for respiration (Burton, 1989; Van der Plas, 1987; Van Es and Hartmans, 1981). Conversion of starch into sugars appears to be reversible (Isherwood, 1973), and when the nett release of sugars from starch exceeds consumption, sugars accumulate. Storage temperature and physiological age of the tuber affect the process of sweetening. Cold storage results in so-called cold-induced sweetening, while ageing of tubers leads to senescent sweetening (Burton, 1989). The storage potential of a certain batch of potatoes is largely determined by the state

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of maturity of the tubers at lifting (Burton, 1965; Coffin et al., 1987; Iritani and Weller, 1980; Nelson and Shaw, 1976; Pritchard and Adam, 1992). Growth conditions and time of harvest are the most important factors affecting this state of maturity.

It will be clear that the final sugar content at processing is the result of several factors influencing the potato during its lifetime. In spite of the apparent differences in storage behaviour, the actual processes leading to accumulation of sugars are likely to be the same for different batches and different cultivars. The differences in degree of accumulation of sugars will solely be the result of the different extent in which the separate processes contribute.

This emphasizes the practical relevance of understanding and describing storage behaviour of different potato cultivars over successive years. A mathematical model describing the accumulation of reducing sugars in potato tubers during storage would substantially assist our understanding of the dynamics of the complex processes leading to sweetening. Such a model was therefore developed, which was subsequently validated by a large set of data on reducing sugar accumulation during potato storage in relation to time and temperature, for different cultivars and seasons. The model itself is based on simplifications of general accepted ideas about the underlying pathways.

2. Materials and methods

2.1. Plant material

Potatoes (*Solanum tuberosum* L. cv. Bintje, Saturna, Agria and Van Gogh) were grown during three successive seasons (1991–1993) at the experimental farm 'De Eest' (Nagele, Noord-Oost Polder, The Netherlands). Plants were grown on clay soil. Each year the seed tubers were planted in early April. Harvest date was different in successive years (Table 1).

2.2. Pretreatment

Tubers of a size suitable for the processing industry were selected for storage experiments by mechanically sorting harvested tubers using a square size of 0.055 m. Potatoes with their smallest diameter larger than 0.055 m were selected. Prior to

Table 1
Plant material used, and storage temperatures applied, during successive storage seasons

	Storage season		
	1991–1992	1992–1993	1993–1994
Cultivars:			
Bintje	+	+	+
Saturna		+	+
Agria			+
Van Gogh			+
Culture:			
Date of planting	March 27	April 10	April 1
Date of harvest	September 25	September 10	October 27
Days to harvest	182	153	209
Storage temperatures:			
2°C	+		+
4°C	+	+	+
5°C			+
6°C	+	+	+
8°C	+	+	+
10°C		+	
14°C		+	+

storage, the potatoes were kept for 14 days at 14°C under sufficient ventilation for drying and wound healing.

2.3. Storage

The different cultivars and storage temperatures used during the successive seasons are given in Table 1. Potatoes were stored in mechanically cooled storage rooms. The tubers were stockpiled using plastic crates each containing 120 tubers of one cultivar. The crates were distributed throughout the storage room to minimize the effect of local differences in temperature, relative humidity and ventilation. Air humidity was controlled by applying a water vapour deficit of 50 Pa.

Storage lasted every year until the end of August. During the first season, sprout growth was suppressed by the chemical sprout inhibitor IPC/CIPC (isopropyl phenylcarbamate/isopropyl *N*-(3-chlorophenyl) carbamate). A sprout inhibitor based on the monoterpene *S*-carvone (D-*p*-mentha-6,8(9)-dien-2-one) was used during the last two seasons (Diepenhorst and Hartmans, 1993; Hartmans et al., 1995; Oosterhaven et al., 1993).

At monthly time intervals throughout the storage period, two sub-samples of 10 tubers each were taken randomly from two crates at different locations in the cold storage room. The 20 tubers were treated as one sample in the sugar determination. During the 1993–1994 season, samples were taken more frequently during the first few months.

2.4. Sample preparation and sugar determination

Tubers were washed and from each tuber one quarter was taken. An aqueous extract was made according to the method of Boehringer Mannheim (1989). To prevent unwanted enzyme activity during extraction, proteins were precipitated using Carrez-I ($K_4[Fe(CN)_6] \cdot 3H_2O$) and Carrez-II ($ZnSO_4 \cdot 7H_2O$). The extracts were stored at $-20°C$. Glucose and fructose were determined enzymatically by a method modified from Viola and Davies (1992) and Boehringer Mannheim (1989). The reagents used were taken from the test combination D-glucose/D-fructose (Cat. No. 139106) from Boehringer-Mannheim (Germany). A serial dilution of glucose was used as a standard. Distilled water was used as a blank. For the glucose/fructose determination, 75 μl of distilled water were added to 50 μl of the sample in a microplate well. Then 75 μl of triethanolamine (Tra) buffer (pH 7.6, containing ATP, NADP and $MgSO_4$ as prepared by Boehringer) were added. The phosphorylation and consecutive oxidation of glucose was initiated by adding of 0.5 units of hexokinase and 0.25 units of glucose-6-phosphate dehydrogenase. Fructose was determined after a subsequent addition of 1.5 units of phosphoglucose isomerase. The NADPH formed during the various reactions was determined spectrophotometrically by measuring its absorbance at 340 nm using a microplate reader. If necessary, samples were diluted. The assay was conducted at ambient temperature. All samples were measured in duplicate. Mean values were used to express the concentration of reducing sugars as %, i.e., g sugar per 100 g fresh weight of tuber tissue.

2.5. Statistical analysis

To be able to statistically analyze the data, an analytic solution of the differential set of equations has been derived using Maple V (release 3.0, Waterloo Maple Software, Canada), a computer program for symbolic mathematical calculation. Eventually the data were analysed statistically with non-linear regression using the statistical package Genstat 5 (release 3.1, Lawes Agricultural Trust, Rothamsted Experimental Station, UK). For each season-cultivar combination separately, the data of all time-temperature combinations were analysed together, using the model formulation of Eq. 9, together with the temperature dependence according to Arrhenius' law (Eq. 6) and with the premises as summarised in Table 2. The data were analysed using simultaneously both time and temperature as explanatory variables. Because of equimolarity, glucose and fructose are considered to be replicates each describing in their own right the accumulation of reducing sugars as a function of time and temperature, thereby doubling the available number of data points for the statistical analysis.

3. Description of the model

3.1. Biochemical formulation and assumptions

Two major phenomena are to be considered in sugar accumulation: cold-induced sweetening and senescent sweetening. In both cases, sugars are released from a large pool of starch. The accumulated sugars may be removed either by respiration or by the resynthesis of starch.

Starch. Considering the large volumes of starch (20%–25% of the fresh weight) compared to the amounts of accumulating sugars (about 1% of the fresh weight), starch can be considered abundantly present and constant throughout the storage period. Hence, starch is not rate limiting.

Cold-induced sweetening. The mechanism of cold-induced sweetening has not yet been conclusively established (Sowokinos, 1990a,b). The sequence by which accumulation of reducing sugars occurs, is mobilisation of starch, followed by an increased synthesis of sucrose and finally hydrolysis of sucrose to glucose and fructose. This last step is affirmed by the almost equimolarity of glucose and fructose during cold-induced sweetening (Claassen et al., 1991; Richardson et al., 1990). Because of the observed equimolarity between glucose and fructose

and their equal relevance for the Maillard reaction, the two forms of reducing sugars are treated as one compound. Sucrose, as a metabolic intermediate, is not explicitly considered in this model. In fact, the overall pathway from starch via sucrose to reducing sugars, is simplified into one step: a conversion from starch to reducing sugars.

Cold-induced sweetening is a reversible process: the level of accumulated sugars decreases with increasing temperatures as a result of increasing sugar consuming reactions such as respiration and resynthesis of starch (Isherwood, 1973; Kim and Lee, 1993; Storey and Briddon, 1993; Williams and Cobb, 1992, 1993). Although the conversion from starch to sugar is realised according to a pathway different from the conversion from sugar to starch, the starch–sugar interconversion can be represented by one reversible reaction. As starch is the main source for maintenance of stored tubers, which continuously demands a certain amount of sugar, the starch–sugar interconversion will always be in the direction of sugar release. The dynamics of this nett release of reducing sugars can finally be simplified and represented by a single irreversible reaction.

Thus, the action of the enzyme system responsible for cold-induced sweetening (indexed cold) is formulated in the model by one enzyme (En_{cold}) that catalyses the release of reducing sugars (S) from starch (Eq. 1).

$$Starch_{n \times S} + En_{cold} \xrightarrow{k_{cold}} Starch_{(n-1) \times S} + En_{cold} + S \tag{1}$$
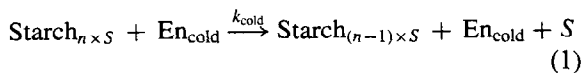
Starch is represented as a polymer consisting of a large number (n) of sugar units (S) that can be hydrolysed one at a time.

During storage over several months, the level of accumulated sugars decreases in general before the onset of senescent sweetening (Burton, 1989). Apparently En_{cold} is susceptible to an increasing malfunctioning during prolonged storage. This may be interpreted as a slow denaturation (indexed *dena*) of the enzyme En_{cold} into a non-active form (indexed *na*) as stated in Eq. 2.

$$En_{cold} \xrightarrow{k_{cold}} En_{cold,na} \tag{2}$$

Senescent sweetening. Sugars mobilised during senescence are released for the benefit of development and growth of sprouts (Burton, 1989). Conse-

quently, senescent sweetening may be initiated soon after the break of dormancy (Burton, 1977; Hughes and Fuller, 1984). As with cold-induced sweetening, senescent sweetening (indexed *sene*) is induced by a second enzyme system (En_{sene}; Eq. 3).

$$Starch_{n \times S} + En_{sene} \xrightarrow{k_{sene}} Starch_{(n-1) \times S} + En_{sene} + S \tag{3}$$
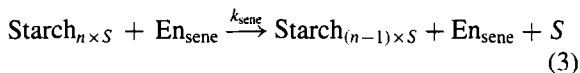
During storage of potatoes for consumption, tuber sprouting is an undesirable phenomenon. It may be suppressed by low temperature storage in combination with a sprout inhibitor. The model is formulated assuming complete sprout inhibition. Inhibition of sprout growth during senescence stimulates the accumulation of reducing sugars (Isherwood and Burton, 1975). Although the external features of sprouting are suppressed, the metabolic processes are apparently not. The higher the storage temperature, the earlier senescent sweetening starts (Barker, 1938). Senescent sweetening does not occur early in storage. As starch is always abundantly present, the amount of enzyme (En_{sene}) responsible for the conversion into sugar has to be very low. To reach an enzyme activity eventually large enough to generate senescent sweetening, the enzyme has to be formed. This increase in En_{sene} is modelled by an exponential formation (indexed *form*; Eq. 4).

$$En_{sene} \xrightarrow{k_{form}} 2 \times En_{sene} \tag{4}$$

This is in agreement with the hypothesis of Kumar and Knowles (1993) who suggest that increased starch hydrolysis during senescence is the result of increasing peroxidative damage of the amyloplast membrane resulting in increasing contact between enzymes and substrate. This also invokes an exponential increase in enzyme activity.

Respiration. During storage, sugars are consumed by respiration. By simple mass action, respiration (indexed *resp*) may be considered to be directly related to the amount of accumulated sugars (Eq. 5). As potatoes are mostly stored under well ventilated atmospheric conditions, the amount of oxygen is considered to be constant (21%) and not rate limiting.

$$S + O_2 \xrightarrow{k_{resp}} CO_2 + H_2O \tag{5}$$

Temperature dependence. Each of the rate constants are assumed to be dependent upon temperature

according to Arrhenius' law:

$$k_i = k_{ref} \cdot e^{\frac{E_i}{R_{gas}} \cdot \left(\frac{1}{T_{ref}} - \frac{1}{T} \right)} \qquad (6)$$

where R_{gas} is the gas constant (8.314 J mol^{-1} K^{-1}). The parameter k_{ref} stands for the reaction rate constant at the reference temperature T_{ref} (K) which was fixed at 279 K (6°C) in the middle of the temperature range studied. The activation energy E_i expresses the dependence of the reaction rate k_i on storage temperature T (K). Formally activation energies have to be positive. If, however, a (bio)chemical equilibrium is simplified into a single irreversible reaction (starch–sugar interconversion), the activation energy of the equilibrium constant is the difference between the activation energies of the forward and backward reaction. This difference can be negative if the backward reaction exhibits a stronger temperature dependence than the forward reaction. So, due to the simplifications made, negative activation energies are allowed.

3.2. Mathematical formulation or dynamic model

The qualitative concept (the five simplified biochemical reactions presented in Eqs. 1–5) can be translated into a quantitative model as a set of differential equations (Eq. 7) by applying the rules of fundamental kinetics. Although Michaelis-Menten kinetics are usually applied in enzyme catalyzed reactions, first order kinetics are used as is argued by Tijskens et al. (1994).

$$\begin{cases} \dfrac{dEn_{cold}}{dt} = -k_{dena} \cdot En_{cold} \\[2ex] \dfrac{dEn_{sene}}{dt} = -k_{form} \cdot En_{sene} \\[2ex] \dfrac{dS}{dt} = k_{cold} \cdot En_{cold} \cdot Starch + \\[1ex] \qquad k_{sene} \cdot En_{sene} \cdot Starch - k_{resp} \cdot S \cdot O_2 \end{cases} \qquad (7)$$

The resulting model can be used for simulations dynamic in time as well as in temperature.

3.3. Analytical formulation or static model

To obtain an analytic solution, the set of differential Eq. 7 was integrated with respect to time at constant temperatures. As a consequence, the deduced analytic solution (Eq. 8) may only be used at constant temperatures.

$$S = \frac{k_{cold} \cdot En_{cold,0} \cdot Starch \cdot \left(e^{-k_{dena} \cdot t} - e^{-k_{resp} \cdot O_2 \cdot t} \right)}{k_{resp} \cdot O_2 - k_{dena}}$$
$$+ \frac{k_{sene} \cdot En_{sene,0} \cdot Starch \cdot \left(e^{k_{form} \cdot t} - e^{-k_{resp} \cdot O_2 \cdot t} \right)}{k_{resp} \cdot O_2 + k_{form}}$$
$$+ S_0 \cdot e^{-k_{resp} \cdot O_2 \cdot t} \qquad (8)$$

The parameter indexed 0 refers to the initial value of that particular parameter. Eq. 8 is subdivided into three terms. The first term describes the nett accumulation of reducing sugars according to cold-induced sweetening. The second term describes in an analogous way the phenomenon of senescent sweetening. The last term describes the consumption of reducing sugars present at the start of storage (S_0) by respiration.

3.4. Premises of the statistical analysis

Prior to the statistical analysis, existing knowledge can be applied for estimating some of the parameters. Furthermore, initial conditions can be set and the sign of the various parameters determined. To begin with, some terms of the model are reparameterized.

The initial values of En_{cold} and En_{sene} always occur in combination with their respective rate constants k_{cold} and k_{sene} and with starch (Eq. 8). Hence, it is impossible to estimate them as separate entities. The product of k_i, $En_{i,0}$ and starch is what is really estimated. By the assumption of abundance, starch can be considered constant during storage. This constant value of starch can therefore be incorporated into the relevant rate constants. The initial values of En_{cold} and En_{sene} are set to the value of 1. In this way the values of En_{cold} and En_{sene} are expressed relative to their initial values while the absolute initial values are also incorporated into the relevant rate constants. As a result the rate constants k'_{cold} and k'_{sene} are now a concatenation of k_i, the absolute initial value of En_i and Starch.

This redefinition of parameters results in an

adapted version of Eq. 8.

$$S = \frac{k'_{cold} \cdot En'_{cold,0} \cdot \left(e^{-k_{dena} \cdot t} - e^{-k_{resp} \cdot O_2 \cdot t}\right)}{k_{resp} \cdot O_2 - k_{dena}}$$
$$+ \frac{k'_{sene} \cdot En'_{sene,0} \cdot \left(e^{k_{form} \cdot t} - e^{-k_{resp} \cdot O_2 \cdot t}\right)}{k_{resp} \cdot O_2 + k_{form}}$$
$$+ S_0 \cdot e^{-k_{resp} \cdot O_2 \cdot t} \tag{9}$$

The initial value for S (S_0) in Eq. 9 is the amount of reducing sugars at the start of storage after the wound healing pretreatment. The model itself can be applied to estimate S_0 using information of preconditioning: 2 weeks at 14°C. This period is recommended for practical conditions in the Netherlands (Meijers, 1981) and is assumed to be long enough to reach a steady state in reducing sugars. At steady state dS/dt (Eq. 6) is zero. As senescent sweetening is not important at this stage, and as En'_{cold} can be assumed to be at its initial value, solving for S results in an expression for S_0.

$$S_0 = \frac{k'_{cold} \cdot En'_{cold,0}}{k_{resp} \cdot O_2} \tag{10}$$

where k'_{cold} and k_{resp} are the rate constants at the temperature of wound healing (14°C).

Because fundamental kinetics require positive reaction rates, all the rate constants and consequently all the reference rate constants are positive. On the base of the model formulation and of the physiological and (bio)chemical meaning of the parameters, signs can be attributed to the activation energies:

Cold-induced sweetening. The reaction leading to cold-induced sweetening from Eq. 1 will be more prevalent at low temperatures than at higher temperatures. This process therefore requires a negative activation energy E_{cold}. The sign of the temperature dependence of the denaturation of En'_{cold} (represented by E_{dena}) can not be fixed in advance.

Senescent sweetening. Both the release of sugars by En'_{sene} and the autocatalytic generation of En'_{sene} are assumed to be stimulated by increasing temperature, thus requiring positive activation energies E_{sene} and E_{form}.

Respiration. The rate of respiration is positively correlated with temperature (the higher the temperature, the higher the respiration; Schippers, 1977a; Schippers, 1977b). Consequently the activation energy E_{resp} has to be positive.

Table 2
Summary of the premises for estimation of parameters using non-linear regression

Parameter	Value	Parameter	Value
$En'_{cold,0}$	1	$k'_{i,ref}$, $k'_{i,ref}$	positive
$En'_{sene,0}$	1	$k'_{sene,ref}$	0.00001
O_2	21	E_{resp}	positive
R_{gas}	8.314	E_{cold}	negative
		E_{dena}	undefined
S_0	$\dfrac{k'_{cold} \cdot En'_{cold,0}}{k_{resp} \cdot O_2}$	E_{sene}	100000
		E_{form}	positive

The number of data points exhibiting senescent sweetening is still limited and hence complicates a meaningful discrimination between the contribution of the separate parameters involved with the process of senescent sweetening. Therefore the number of parameters to be estimated is limited by fixing the parameters $k'_{sene,ref}$ and E_{sene} at the respective values 0.00001 and 100 000, being reasonable values as derived from prior analysis trials.

4. Experimental results

4.1. Glucose/fructose ratio

The reducing sugars glucose and fructose accumulated in more or less equal portions during storage. In Fig. 1, the data of successive years and various storage temperatures are combined. The cultivar Saturna shows a low degree of cold susceptibility; most of the data points are clustered in the region of low sugar concentrations. The few points at higher sugar concentrations exert a high leverage on the slope of the regression line, resulting in a slope for Saturna somewhat dissimilar to that of the other three cultivars. The slope of the regression line is equivalent with the ratio glucose/fructose. No systematic differences between the four cultivars could be found with regard to this ratio in which glucose and fructose accumulated. The slope for the entire set of data of the four cultivars was 0.94. This validates the assumption of equimolarity of glucose and fructose, and allows the use of both components as expressions of the same variable to be analysed.

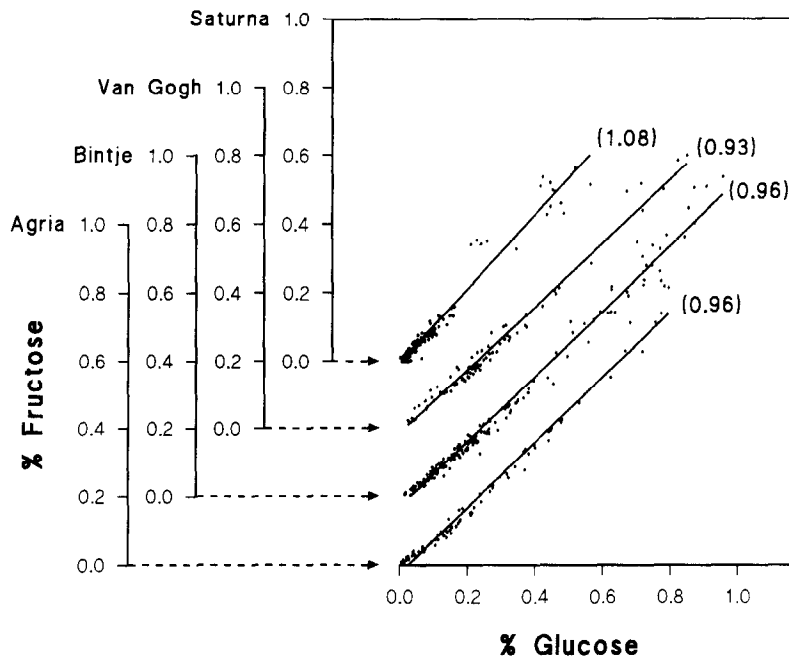Accumulation of reducing sugars as a function of

Fig. 1. Relation between the concentration of glucose and fructose during storage of four potato cultivars (Agria, Bintje, Van Gogh and Saturna). To clarify the possibly different behaviour of different cultivars, the axes are shifted from each other. Data from the successive years are combined. The slopes of the four regression lines are given in parentheses.

time and temperature for the four cultivars over successive storage seasons is given in Fig. 2. The initial amount of reducing sugars present in Bintje tubers was considerably higher for the 1993–1994 storage season than for the earlier two seasons (Fig. 2C). The initial sugar concentration for Agria and Van Gogh in 1993–1994 was also high (Fig. 2F and G).

4.2. Cold-induced sweetening

During the first hundred days of storage for each of the season-cultivar combinations, reducing sugars accumulated more the lower the storage temperature. Cold-induced sweetening occurred particularly at temperatures below 8°C. The level of sugars accumulated varied both with cultivar and with season. As was already seen in Fig. 1, Saturna (Fig. 2D and E) showed a very low degree of cold susceptibility resulting in relative low levels of reducing sugars whereas the other three cultivars were evidently more susceptible to cold storage as they showed a marked accumulation of reducing sugars. Storage of Bintje

tubers at 4°C during the 1993–1994 season (Fig. 2C) resulted in low levels of reducing sugars (0.65%) by comparison with an accumulation of 1.2% at 4°C during the 1991–1992 season (Fig. 2A) and 1.5% during the 1992–1993 season. During storage, the levels of accumulated sugars reduced gradually (e.g., Fig. 2A). The effect of temperature on the accumulation of reducing sugars was not linear. The effect of a fixed difference in storage temperature (for example 2°C) became greater with lower temperatures (e.g., Fig. 2G).

4.3. Senescent sweetening

Senescent sweetening only occurred during two storage seasons of Bintje (Fig. 2B and C) and to a minor extent during one storage season of Saturna (Fig. 2D). At higher storage temperatures (8°C–14°C for Bintje and 10°C–14°C for Saturna), the level of reducing sugars gradually increased towards the end of storage. The higher the storage temperature, the sooner senescent sweetening started and the faster it developed. During prolonged storage at 14°C (after
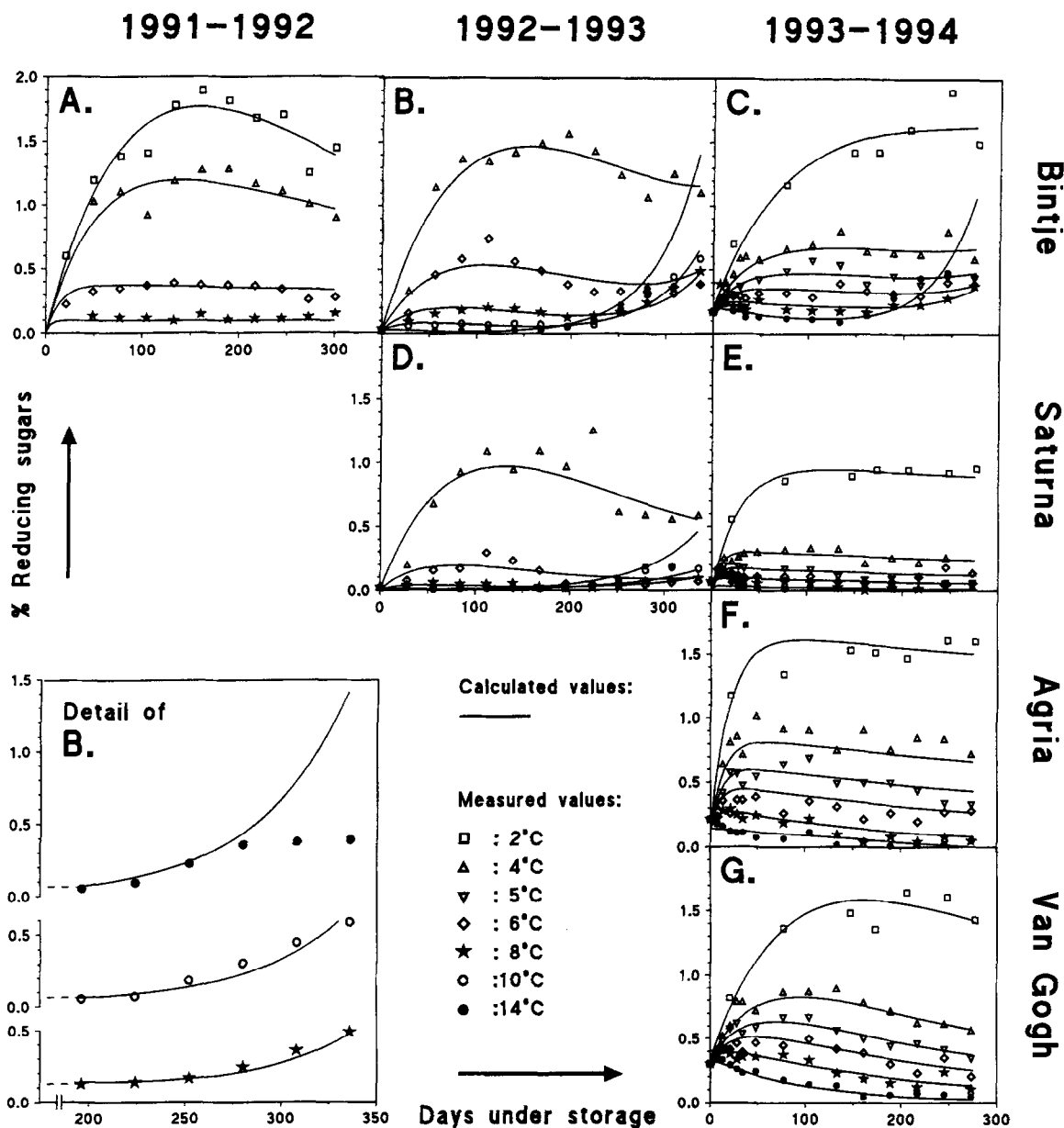
Fig. 2. Accumulation of reducing sugars during storage of the potato cultivars Bintje, Saturna, Agria and Van Gogh over successive seasons. Storage temperatures ranged from 2 to 14°C. In the detail of B, the last 150 days of storage under 8°C, 10°C and 14°C are presented with their y-axes shifted from each other. Symbols are the measured values, the solid lines represent the calculated values according to the model.

300 days) sprouting pressure became too large to maintain complete sprout inhibition (data not given). In these cases senescent sweetening decreased again (see detail of Fig. 2B). At 8 and 10°C, with no excessive sprouting, reducing sugars kept accumu-

lating. Data points that showed a decrease in the accumulation of reducing sugars during senescent sweetening were left out of the statistical analysis as the assumption of complete sprout inhibition was no longer valid.

For the other season-cultivar combinations, senescence might already have been in progress, but extra accumulation of reducing sugars at higher storage temperatures was not observed.

5. Model results

The results of the non-linear regression analysis of each of the season-cultivar combinations using time and temperature simultaneously as explanatory variables, are presented in Table 3. The goodness of fit of the model is expressed by R^2_{adj}. If the data did not show senescent sweetening, it was inherently impossible to estimate the model parameters of senescent sweetening. In these cases the model was simplified by fixing $En'_{sene,0}$ to a value of zero, thereby removing the process of senescent sweetening completely. So the parameters for senescent sweetening could only be estimated for the data of two seasons of Bintje (1992)–1993, 1993–1994) and of one season of Saturna (1992)–1993). The simulated data, generated by the model applying the parameters estimated are shown in Fig. 2 as solid lines.

Notwithstanding the fact that each of the season-cultivar combinations exhibits its own pattern of sugar accumulation, the developed model is capable of describing 95% of the observed storage behaviour.

6. Discussion

6.1. Storage behaviour

The fact that the glucose:fructose ratio remained constant for all cultivars over all seasons proves that the mechanism for production of these sugars, the hydrolysis of sucrose, does not change during storage at any time at any temperature.

The high initial concentration of reducing sugars during the 1993–1994 season is a clear seasonal effect. Due to unfavourable weather conditions prior to harvest the tubers were exposed to low soil temperatures. Because of the incurred cold-induced sweetening during this period in the soil, the assumption that the pretreatment period at 14°C was long enough to reach a steady state in reducing sugars might not have been valid during the 1993–1994 season. This is confirmed by the decrease in reducing sugars during continuing storage at 14°C.

Although each season-cultivar combination exhibited throughout the storage a different pattern of accumulation of reducing sugars, the developed model explains the observed behaviour for about 95%. This confirms that the processes leading to accumulation of sugars are on principle the same for all described cases, and that the observed differences in storage behaviour are solely the result of the different amplitudes of the separate processes.

Table 3
Non-linear regression analysis of reducing sugar accumulation versus storage time

Parameter [a]	Bintje			Saturna		Agria	Van Gogh
	1991–1992	1992–1993	1993–1994	1992–1993	1993–1994	1993–1994	1993–1994
$k'_{cold,ref}$	0.0127	0.00662	0.00420	0.00330	0.0160	0.0165	0.00588
E'_{cold}	−29542	−201320	−143317	−353462	−12716	−128436	−109872
$k'_{dena,ref}$	0.000508	0.00874	0.0422	0.0145	0.00233	0.0958	0.0368
E'_{dena}	−445723	104278	202165	220697	242640	110598	182495
$k'_{form,ref}$	n.a. [b]	0.0175	0.0177	0.0123	n.a.	n.a.	n.a.
E'_{form}	n.a.	16482	19050	28026	n.a.	n.a.	n.a.
$k'_{resp,ref}$	0.00312	0.000450	0.0000839	0.000494	0.0153	0.000109	0.000174
E'_{resp}	423843	117157	119579	86141	356316	254499	79687
R^2_{adj}	96.7%	96.1%	93.8%	94.1%	94.2%	94.6%	95.9%
n	92	126	164	123	166	166	166

[a] $k_{i,ref}$ = reaction rate constant at reference temperature T_{ref} (=6°C); E_i = activation energy of reaction rate constant k_i; R^2_{adj} = percentage variance accounted for; n = number of data points.
[b] n.a. = not applicable.

Only for the storage of Agria at 4°C (Fig. 2F) is the actual amount of sweetening underestimated by the model. This can not be a consequence of a deviant (too low) storage temperature, because in that case it would also have effected the other cultivars stored in the same cold storage room.

When excessive sprouting occurs during prolonged storage after 300 days at 14°C, resulting in a decrease in accumulation of reducing sugars (as can be seen in the detail of Fig. 2B), the model formulation is no longer valid. The model simulates as if sprout inhibition is complete. The developing sprouts form, in fact, an additional sink, removing sugars from the tuber and thus being responsible for the observed change in senescent sweetening. At 8°C and 10°C, when no excessive sprouting occurred, the model correctly describes senescent sweetening.

6.2. Seasonal variations

As can be seen in Table 3, the seasonal effect on the cultivars was not limited to one specific parameter. The applicability of the developed model, however, would expand considerably if one or more parameters can be interrelated with certain cultivar or season specific features.

As already stated, harvest date differed over the successive years, resulting in various states of maturity. During the development of tubers attached to the mother plant the activity of several enzymes related to sugar metabolism (such as sucrose synthase, ADP-glucose pyrophosphorylase and ATP fructokinase) change (Merlo et al., 1993; Morrell and Rees, 1986). Merlo et al. (1993) reported for most enzyme activities in developing tubers, an optimum curve with the optimum for 8-week-old plants. The observation of Merlo et al. implies that the initial value of En'_{cold} ($En'_{cold,0}$) depends on the state of maturity at harvest. Based on this reasoning, the combined data of all storage seasons of Bintje and Saturna were re-analysed, attributing all seasonal effects to only one variable: $En'_{cold,0}$. The remaining parameters were estimated in common for the successive seasons and became cultivar specific. As $En'_{cold,0}$ is a relative value, and up to now fixed at 1, some reference point had to be chosen. For this purpose the value of $En'_{cold,0}$ in the 1992–1993 season, which
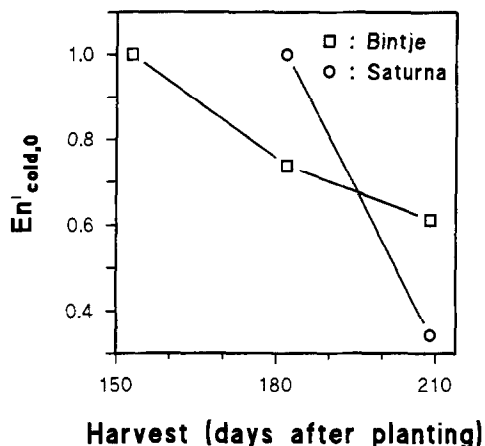

Fig. 3. Estimations of the season-specific parameter $En'_{cold,0}$ for Bintje and Saturna tubers in relation to the length of the growth period.

probably generated the most immature tubers, was fixed at 1 as a standardised reference for the other seasons. If the observed seasonal variation is in this way attributed to one parameter, the model is still capable of explaining 92% of the combined data of Bintje and 92% of the combined data of Saturna.

The values estimated for $En'_{cold,0}$ for the individual seasons appeared to depend on the length of the period from planting to harvest (Fig. 3). This is in agreement with the observations described by Merlo et al. (1993). Although the maturity of tubers is not exclusively determined by the length of the period from planting to harvest, it was a dominant factor influencing the state of maturity during the successive seasons.

7. Further validation

The effect of different maturity stages on the accumulation of reducing sugars during storage was studied by Putz (1993). The average data over ten potato varieties were presented. Tubers were harvested weekly (July 7 to August 29) and were stored for 16 weeks at 4°C without applying a pretreatment for wound healing. A part of the measured data on reducing sugars are represented in Fig. 4 as symbols. The later the tubers were harvested, the lower the initial level of reducing sugars and the lower the subsequent maximum accumulation reached. No seasonal variation is included in the data of Putz, so
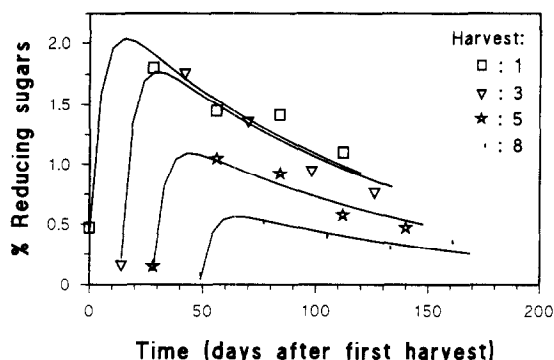
Fig. 4. Accumulation of reducing sugars average for ten potato cultivars. The cultivars were harvested weekly and were stored for 16 weeks at 4°C. The numbers refer to the successive harvests. Symbols represent data as published by Putz (1993), the solid lines represent the calculated values according to the model ($R^2_{adj} = 97.2\%$). Only data of four of the harvest dates are shown.

Fig. 5. Estimations of $En'_{cold,0}$ for the data of Putz (1993) in relation to time of harvest relative to the first harvest.

the state of maturity at harvest is directly related to the time of harvest.

To analyze this set of data using the approach described above a few minor adaptations were made. Due to the single temperature applied in the experiment, no temperature dependence could be estimated, so k_i (at 4°C) was estimated directly. The initial values of reducing sugars (S_0) for the eight harvest dates were estimated by an exponential decrease in reducing sugars during the 8 weeks of development of the tubers attached to the plant. Again as a reference, $En'_{cold,0}$ of the first harvest was fixed at a value of 1. Due to the short period of storage, senescent sweetening could be neglected, so $En'_{sene,0}$ was fixed at zero.

The effect of the different maturity stages on the accumulation of reducing sugars during storage can be explained at the 97.2% level. The initial amounts $En'_{cold,0}$ and S_0 are specific for the successive harvest dates, while the other model parameters are estimated in common for the combined set of data. The simulated data generated by the model with the parameters estimated, are for four of the eight harvests shown in Fig. 4 as solid lines. The estimated initial values $En'_{cold,0}$ versus time of harvest are shown in Fig. 5. $En'_{cold,0}$ again decreases almost linearly as a function of harvest time and thus of the related state of maturity. This analysis of an independent set of data confirms the concept that the state of maturity at time of harvest determines the storage behaviour
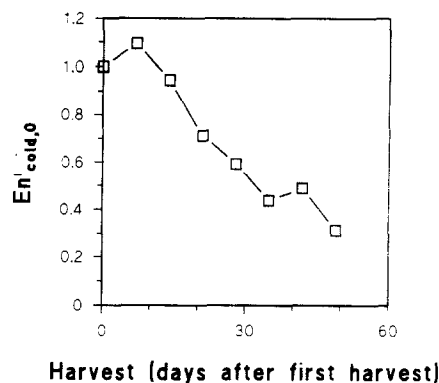
through the initial amount of enzyme (or enzyme system) responsible for cold-induced sweetening.

8. Conclusion and prospects

In spite of the simplifications made, our model is capable of describing 95% of the storage behaviour in terms of the accumulation of reducing sugars of several cultivars over a number of years. The concept is postulated that the state of maturity at time of harvest determines storage behaviour through the amount of enzyme (En_{cold}) present. This hypothesis is validated by an independent set of data. It is not likely that the model enzyme En_{cold} can be traced as one concrete enzyme. Probably, En_{cold} must be seen as a complex of metabolic activity. However, for practical purposes it is not necessary to identify the model enzyme En'_{cold} as a single enzyme. When a maturity index can be defined as biochemical and/or physical properties of tubers at the time of harvest, and can be correlated with the model parameter $En'_{cold,0}$, it becomes possible to use the model for predicting storage behaviour of a certain batch of potatoes. For a further expansion of the applicability of the model, insight is needed in the physiology underlying cultivar specific differences. It will be clear that a subsequent stage of the model must be able to handle the effect of sprouting in relation to (senescent) sweetening.

Acknowledgements

List of symbols

E	activation energy
En	enzyme
k	reaction rate constant
S	reducing sugars
t	time
T	temperature

Indices

0	initial amount
cold	during cold storage
dena	denaturation
form	formation
I	an arbitrary index
na	not active
ref	reference
resp	respiration
sene	during senescence

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